

Elevated Serum Concentrations of Cardiac Troponin T in Acute Allograft Rejection After Human Heart Transplantation

THOMAS J. DENGLER, MD,* RAINER ZIMMERMANN, MD,* KLAUS BRAUN,*
MARGIT MÜLLER-BARDORFF, MD,§ JÖRG ZEHELEIN, MD,* FALK-UDO SACK, MD,†
PHILIPP A. SCHNABEL, MD,‡ WOLFGANG KÜBLER, MD,* HUGO A. KATUS, MD§

Heidelberg and Lübeck, Germany

Objectives. This study evaluates the concept and diagnostic efficacy of using serum troponin T for the detection of cardiac graft rejection.

Background. Cardiac troponin T is a cardiospecific myofibrillar protein, which is only detectable in the circulation after cardiac myocyte damage. It might be expected to be released during acute heart allograft rejection, allowing noninvasive rejection diagnosis.

Methods. In 35 control subjects and in 422 samples from 95 clinically unremarkable heart allograft recipients more than 3 months postoperatively, troponin T serum concentrations were compared to the histological grade of acute graft rejection in concurrent endomyocardial biopsies.

Results. Mean troponin T serum concentrations were identical in control subjects (23.2 ± 1.4 ng/liter) and in heart transplant recipients without graft rejection (International Society for Heart and Lung Transplantation [ISHLT] grade 0; 22.4 ± 1.7 ng/liter). Mean troponin T concentrations increased in parallel with the severity of graft rejection (ISHLT grade 1: 27.8 ± 1.8 ng/liter; grade 2: 33.2 ± 2.7 ng/liter; grade 3A: 54.6 ± 6.5 ng/liter; grade 3B

and 4: 105.4 ± 53.7 ng/liter; $p < 0.001$ for grades 3 and 4 vs. grades 0 and 1). The proportion of positive samples also increased in parallel with rejection severity, reaching 100% in rejections of grade 3B and 4. Sensitivity and specificity for the detection of significant graft rejection (ISHLT grade 3/4) were 80.4% and 61.8%, respectively. The negative predictive value was most remarkable with 96.2%. Intraindividual longitudinal analysis of troponin T levels and biopsy results in 15 patients during long-term follow-up confirmed these findings.

Conclusions. The present data demonstrate that acute allograft rejection after human heart transplantation is often associated with increased serum concentrations of troponin T. All cases of serious forms of graft rejection would have been detected before the development of clinical symptoms. Measurement of troponin T levels may become a useful ancillary parameter for noninvasive rejection diagnosis, being most valuable in the exclusion of severe cardiac graft rejection.

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The cardiac myofibrillar protein troponin T has several potential advantages in the diagnosis of myocardial cell damage over the measurement of more traditional serological markers such as lactate dehydrogenase (LDH) or creatine kinase (CK). These include its expression as a strictly cardiospecific isoform, the high intracellular concentration and rapid release upon cell damage (1-4). A specific enzyme immunoassay is available for the determination of circulating cardiac troponin T, showing very little cross-reactivity with troponin T isoforms from skeletal muscle (3,5). With current commercial immunoassays, circulating cardiac troponin T is only detectable in the pres-

ence of cardiac muscle damage as in severe cases of unstable angina, myocardial infarction or after cardiac surgery (4,6,7).

Recently, we and others described continued release of cardiac troponin T over 2 to 3 months after heart transplantation (1,2,8), outlasting troponin T release following myocardial infarction or standard cardiac surgery, the exact cause being still unclear. In some cases, episodes of early postoperative rejection seemed to be accompanied by slight rises in troponin T serum levels, overshadowed mainly, however, by the high troponin T background concentrations (8).

Acute allograft rejection after heart transplantation is characterized by immune-mediated myocardial cell destruction; it therefore appears conceivable that rejection episodes should lead to elevated serum troponin T concentrations through release from damaged cells, detectable mainly after cessation of the early posttransplant release described above. Preliminary experimental animal data by Walpoth et al. (9) and ourselves (unpublished data) suggest such an increase in serum troponin T levels in severe rejection. Despite continuing efforts in serology and with alternative methods (magnetic resonance imaging [MRI] [10], signal-averaged electrocardiogram [ECG] [11], specialized echocardiographic indices [12,13], cytoimmu-

From the *Department of Cardiology, †Department of Cardiac Surgery, and ‡Department of Pathology, University of Heidelberg, Heidelberg, Germany; and §Department of Cardiology, University of Lübeck, Lübeck, Germany. This study was supported in part by a research grant to H.A. Katus by the Medical Faculty and Transplant Center of Heidelberg University.

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Address for correspondence: Thomas J. Dengler, MD, Boyer Center for Molecular Medicine, Yale School of Medicine, 295 Congress Ave, room # 449, New Haven, Connecticut 06510. E-mail: thomas.dengler@yale.edu

Abbreviations and Acronyms

CK	= creatine kinase
ECG	= electrocardiogram
ELISA	= enzyme-linked immunosorbent assay
ISHLT	= International Society for Heart and Lung Transplantation
LDH	= lactate dehydrogenase
MRI	= magnetic resonance imaging

nological monitoring [14]), there are currently no reliable and easily available parameters for routine noninvasive rejection monitoring after heart transplantation.

Classic markers of myocyte injury like CK and its MB isoform were found not to be sufficiently sensitive (15-17). Therefore, a sensitive cardiospecific marker indicating myocyte damage due to allograft rejection could be a useful adjunct in noninvasive rejection diagnosis after cardiac transplantation. The present study evaluates the use of serum troponin T measurements with optimized sensitivity for the detection of ongoing acute allograft rejection in humans.

Patients and Methods

Controls. The control group comprised 35 healthy blood donors (20 male, 15 female, mean age: 35.4 ± 15.2 years, range: 19-52).

Heart transplant patients. Ninety-five heart transplant recipients were studied; clinical data are shown in Table 1. Only patients beyond 3 months after operation were included in the

study. Patients gave written consent, and the study was approved by the local institutional ethics committee. Exclusion criteria were signs of graft failure (clinical examination, routine echocardiography, electrocardiogram, hemodynamic compromise), severe transplant atherosclerosis with significant lesions on coronary angiograms, renal failure (serum creatinine concentration >4 mg/dl) or cytomegalovirus infection involving the myocardium (evidenced by positive immunostaining). Patients were treated with standard triple drug immunosuppression with cyclosporine A, corticosteroids and azathioprine, azathioprine being withheld with blood leukocyte counts below $4000/\text{mm}^3$ ($4/\text{nl}$); details of immunosuppression are shown in Table 1. In all patients, endomyocardial biopsy results and serum samples were available concurrently. Biopsy material was obtained as part of routine posttransplant care; endomyocardial biopsies were performed at routine intervals; histological rejection diagnosis was graded according to the nomenclature by the International Society of Heart and Lung Transplantation (ISHLT) of 1990 (18). As the study was performed in the years 1992 to 1995, the diagnosis of ISHLT grade 2 rejection was included in biopsy evaluation and data analysis. In all cases of cellular infiltration compatible with a diagnosis of rejection \geq ISHLT grade 2 immunostaining for cytomegalovirus antigen pp65 was performed (anti-CMV antibody E-13, Paesel und Lorei, Frankfurt, Germany) to exclude cytomegalovirus myocarditis. Acute rejection episodes were treated with 3×1 g of intravenous methylprednisolone or 6×100 mg of oral methylprednisolone, based on clinical decision; 1 patient required an additional 3-day course of antithymocyte globulin (5 mg/kg body weight per day) for steroid refractory rejection (ISHLT grade 4). No patient included in the study received methotrexate, OKT-3 or FK506.

Troponin T concentrations were measured in available serum samples from all cases of serious acute graft rejection (ISHLT grades ≥ 2) between June 1992 and June 1995 ($n = 144$) and in 271 samples of patients with no or only mild allograft rejection, matched for similar time after transplantation. In the study period, 7 cases of severe, stenosing immune vasculitis without myocardial cellular infiltrates were noted. These could not be graded by ISHLT nomenclature, but they were clinically regarded as significant rejection, treated accordingly and included in the study for troponin T determination as a separate group.

Longitudinal analysis. In 15 long-transplanted patients (13 male, 2 female, age: 49.5 ± 27.1 years [range: 24-63], preoperative diagnoses: dilated cardiomyopathy: $n = 9$, ischemic heart disease: $n = 6$) an intraindividual longitudinal analysis comparing serial serum troponin T measurements with histological rejection grades was performed. The mean length of postoperative study was 695 ± 672 days (range: 370-1213) with a total of 23 rejection episodes (ISHLT grade ≥ 3 , median per patient = 1, range 0-2). One hundred sixty-eight biopsies and troponin serum levels were evaluated (median per patient: 10, range 5-20).

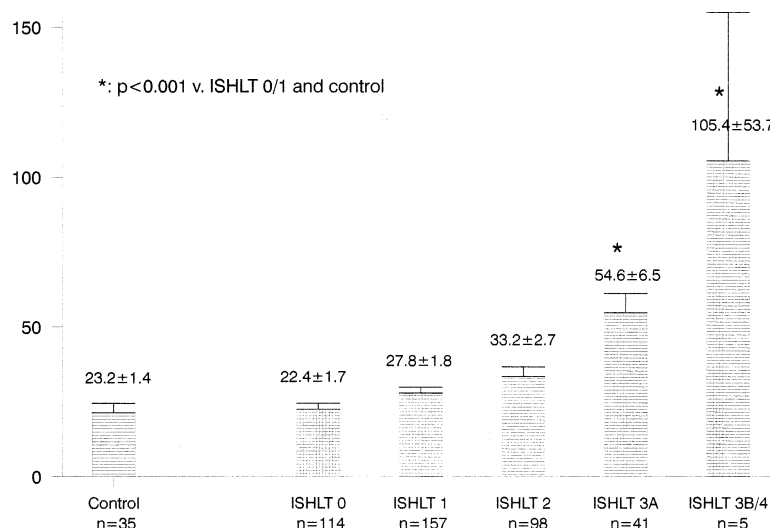
Enzyme-linked immunosorbent assay (ELISA). Troponin T concentrations were measured in an optimized one-step

Table 1. Heart Transplant Recipients: Demographic and Clinical Data

Male/female (n)	78/17
Number of biopsies/serum samples per patient (n [median (range)])	4 (1-7)
Age (years [mean (SD), range])	48.2 (11.4), 18-63
Time after transplantation* (days [median (range)])	
All	470 (93-2100)
Grade 0†	518 (93-1935)
Grade 1	388 (94-2100)
Grade 2	501 (96-1998)
Grade 3A	360 (95-2085)
Grade 3B/4	176 (120-501)
Preoperative diagnosis (n)	
Dilated cardiomyopathy	60
Ischemic heart disease	30
Other	5
Cyclosporine blood level ($\mu\text{g/liter}$ [weighted mean \pm SD (range)])	237 ± 74 (160-324)
Daily steroid dose (mg [mean \pm SD (range)])	
First postoperative year (n = 95)	14.8 ± 5.3 (5-30)
>1 year postoperative (n = 81)	7.3 ± 2.4 (3.5-10)
Daily azathioprine dose (n = 67) (mg [mean \pm SD, (range)])	59.4 ± 44 (25-150)

*Mean time after transplantation refers to all 422 serum samples/endomyocardial biopsies. †Nomenclature of the International Society of Heart and Lung Transplantation for rejection severity.

Figure 1. Mean troponin T serum concentrations (\pm SEM [ng/liter]) in separate groups of ISHLT rejection grade versus controls *Significant differences ($p < 0.001$) versus controls and ISHLT grade 0/1 rejection.



ELISA based on the Enzygum-Test Troponin T (Boehringer Mannheim, Mannheim, Germany). A biotinylated troponin T antibody (1B10) was precoated to a streptavidin-covered microtiter well plate. Fifty microliters of sample were added with 200 μ l of a second cardiospecific troponin T antibody (M7) covalently linked to peroxidase in incubation buffer (0.1 mol/liter sodium citrate, 0.47 mol/liter Na_2HPO_4 , 0.1% bovine serum albumin at pH 6.3). After 40 min incubation, plates were washed three times, then 250 μ l of ATBS (Boehringer Mannheim, Mannheim, Germany) substrate were added. After 60 min incubation, microtiter plates were read at 405 nm on an microtiter reader photometer (SLT, Crailsheim, Germany). For quantitation, linear standard curves were established over the required concentration range using bovine troponin T standards supplied by the manufacturers.

Data analysis. For comparison of continuous data, troponin T determinations below the detection limit were assigned the concentration of the detection limit (15 ng/liter) in order to rather underestimate differences between groups. In addition, the proportion of positive troponin T determinations was compared between patient groups using the detection limit (15 ng/ml) as cutoff level for positive versus negative troponin T determinations. Differences between patient groups were evaluated by analysis of variance, followed by the Bonferroni *t* test or chi-square test as applicable. Comparison of serum troponin T with clinical and laboratory variables was done by linear regression analysis or *U* test. Statistical significance was assumed for $p < 0.05$; data analysis was performed using the SAS statistical software package.

Results

ELISA. The ELISA used for this study was optimized for greater sensitivity compared to currently available commercial assays by sequential step performance in streptavidin-coated microtiter plates and longer incubation times. The detection limit was experimentally determined according to Kaiser (19);

photometric absorption of blank values ($n = 12$) was 0.161 ± 0.0032 (mean \pm 1 SD), with 17 ng/liter of troponin T ($n = 12$) absorption was 0.184 ± 0.0042 . Extrapolating from the gradient of the troponin T standard curve, the detection limit was determined at 15 ng/liter. The intraassay and interassay coefficients of variation were 2.3% and 6.4%, respectively.

Control subjects. In the control subjects, mean troponin T concentrations were 23.2 ± 1.4 ng/ml (Fig. 1); the proportion of positive troponin T tests in the control subjects was 25.7% (Fig. 2).

Cardiac troponin T levels in heart transplant patients. Troponin T serum levels were measured in 422 blood samples of 95 heart transplant recipients, including all cases of more severe allograft rejection (ISHLT grades ≥ 3). The distribution of rejection grades and timepoints of determination is shown in Table 1 and Figure 1. The temporal relation of the different rejection grades to transplantation was similar in all groups; only very severe graft rejections (ISHLT grade 3B and 4) occurred earlier after transplantation (Table 1). Based on the exclusion criteria of the study, all transplant patients had normal graft function on routine echocardiography. Cases of confirmed or potential cytomegalovirus infection of the myocardium by immunohistology ($n = 2$) were not included in the analysis.

In patients without graft rejection (ISHLT grade 0), mean serum concentrations of troponin T were identical to control levels (22.4 ± 1.7 ng/liter vs. 23.2 ± 1.4 ng/liter, $p = \text{NS}$; Fig. 1). In transplant patients with graft rejection, mean troponin T concentrations increased in parallel with the severity of graft rejection (mean \pm SEM; ISHLT grade 1: 27.8 ± 1.8 ng/liter; grade 2: 33.2 ± 2.7 ng/ml; grade 3A: 54.6 ± 6.5 ng/liter; grade 3B and 4: 105.4 ± 53.7 ng/liter; Fig. 1). The highest troponin T level occurred in the only case of grade 4 rejection (318 ng/ml). For ISHLT grades 3 and 4, the differences were statistically significant versus controls and ISHLT rejection grades 0/1 ($p < 0.001$) and also versus ISHLT grade 2 ($p < 0.05$). Troponin T

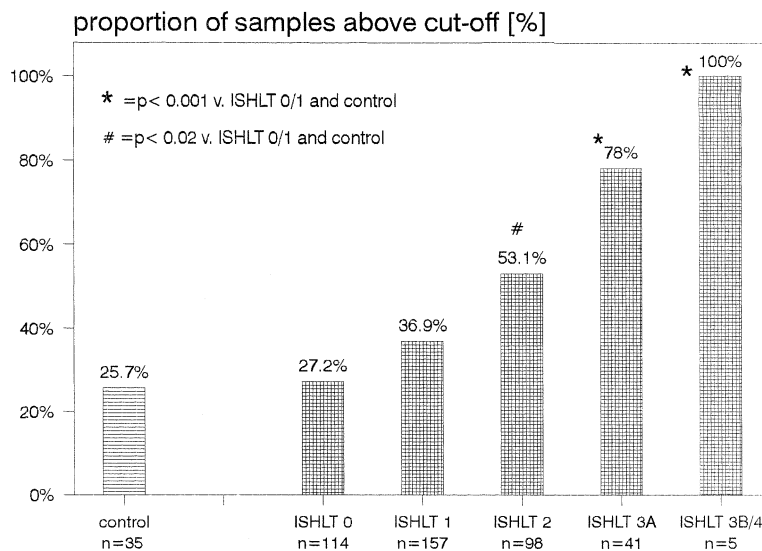


Figure 2. Proportion of positive troponin T determinations (levels above cutoff at 15 ng/ml) in separate groups of rejection grade and controls. *Significant difference versus controls and ISHLT grade 0/1 rejection: $p < 0.001$; # = ditto, with $p < 0.02$.

levels in grade 1 and 2 rejection were not significantly different from controls (Fig. 1).

Using the detection limit of the assay at 15 ng/liter as a diagnostic cutoff level, the proportion of positive samples (above cutoff) also increased in parallel with rejection severity, reaching 78% in grade 3A rejection and 100% in grade 3B and 4 rejection (32/41 and 5/5 cases, respectively; Fig. 2). The proportion of positive samples in cases of rejection with ISHLT grade 3A and 3B/4 was significantly higher than in cases of no or mild rejection (ISHLT 0–1; $p < 0.001$; Fig. 2). With grade 2 rejection, the proportion of positive samples was also higher than in cases of no or mild rejection, but on a much lower level of significance ($p < 0.02$). Again there was no significant difference between control subjects and patients with no or mild rejection (25.7% vs. 27.2% and 36.9%, $p = \text{NS}$).

Comparison of all cases of more severe forms of rejection (ISHLT grades 3 and 4) with lower rejection grades (ISHLT grades 0–2) showed significantly higher mean troponin T serum levels in serious rejection with 60.1 ± 8.5 ng/liter versus 27.6 ± 2.0 ng/liter, $p < 0.001$. The proportion of positive troponin T determinations was also significantly higher in severe forms of rejection (80.4% vs. 38.2%, $p < 0.001$). From these data, sensitivity and specificity of troponin T determinations for the detection of serious rejection (ISHLT grades 3 and 4) were determined at 80.4% and 61.8%, respectively. The negative predictive value of the troponin T test was 96.2% (absence of a serious form of allograft rejection with troponin T concentration below the diagnostic cutoff).

During the study period there were 7 cases of severe stenosing immune vasculitis without any myocardial cellular rejection (isolated vasculitic rejection) and qualitatively unimpaired left ventricular function. Mean troponin T serum concentrations of these cases were significantly increased versus control levels or levels in cases with rejection of ISHLT grade 0 or 1 (42.9 ± 9.0 ng/liter; $p < 0.01$). In all 7 cases the serum troponin T levels were above the diagnostic cutoff (100%).

Troponin T levels were not correlated with the time after transplantation, either in an overall analysis ($r = -0.053$, $p = \text{NS}$) or when limited to severe cases of graft rejection (ISHLT 3 and 4; $r = -0.104$, $p = \text{NS}$). Troponin T serum concentrations were not significantly correlated with any other clinical or laboratory variables. There were no cases of severe systemic infections among the study patients; minor flu-like illnesses and bacterial pneumonia ($n = 3$) in the absence of allograft rejection did not affect troponin T serum concentrations (not shown). Serum creatinine concentrations as a marker of renal excretory function were not correlated with troponin T concentrations ($r = 0.078$, $p = \text{NS}$); also recipient or donor age, gender and liver function parameters showed no association.

In all serum samples, CK serum concentrations were determined in parallel; in only 8 cases CK levels above the upper limit of normal (80 U/liter) were detected ranging from 85U/liter to 224U/liter without any correlation to allograft rejection. Overall, CK serum concentrations were not significantly different between cases of severe rejection (ISHLT grade 3 + 4) and those of no or mild forms of rejection (ISHLT 0–2) with 33.2 U/liter versus 31.4 U/liter ($p = \text{NS}$). No association of CK levels with rejection severity or troponin T levels could be demonstrated. CK-MB isoforms were not investigated.

Longitudinal studies. In 15 long-term transplant patients an intraindividual longitudinal analysis was performed retrospectively on 168 biopsies and corresponding serum samples for troponin T determination. Due to the doubtful significance of ISHLT grade 2 rejections ($n = 4$, troponin T elevation in 2/4), these were excluded from this analysis. Twenty-three episodes of advanced rejection (ISHLT grade ≥ 3) were diagnosed within the study period; 34 samples with elevated troponin T concentrations were identified. A summary of the results of the longitudinal comparison of troponin T levels and histological rejection grades is presented in Table 2.

In 21 of 23 episodes (91.3%) of significant acute graft rejection a concordant rise of troponin T serum concentrations

Table 2. Square Table Analysis of Retrospective Intraindividual Longitudinal Comparison of Troponin T Serum Concentrations Measured by ELISA and Histological Rejection Grades in Concomitant Myocardial Biopsies in 15 Heart Transplant Recipients During Long-Term Follow-up

	Troponin T positive (concentration above cutoff [15 ng/ml])	Troponin T negative (concentration below cutoff)	Total
Rejection positive (ISHLT grade ≥ 3)	21	2	23
Rejection negative (ISHLT grade < 3)	13	128	141
Total	34	130	164

Median number of biopsies per patient: 10 (range: 5-20), mean length of follow-up: 695 days (range: 370-1213).

with rejection and subsequent decline during recovery (usually by increased antirejection therapy) was found. A concordant course of troponin T levels was assumed if, after a rejection-free period with stable troponin T levels below the detection limit, troponin T concentrations rose above the detection limit during a rejection episode and returned to prerejection levels with or soon after histological recovery. In the remaining 2 cases (8.7%), rejection episodes (ISHLT grade 3A) occurred without definite increases in troponin T concentrations (false negatives); both these incidents occurred very late after transplantation (45 and 100 months). In 13 of 34 cases (38.2%) with increases in troponin T serum levels no concomitant histological evidence of graft rejection was found (false positives); in 128 of 145 cases without significant rejection (88.3%) no significant rise in troponin T was detectable (true negatives). On the basis of these data, sensitivity and specificity of the longitudinal troponin T analysis were calculated at 91.3% and 88.3%, the negative predictive value being $>98\%$. In 2 additional patients, there was unexplained, continued release of troponin T without evident clinical symptoms. Figure 3 shows 2 examples of concordant behavior of troponin T levels and rejection severity, among them the only case (EP) of ISHLT grade 4 rejection requiring therapy with antithymocyte globulin displaying markedly elevated troponin T levels.

Discussion

In contrast to liver or kidney transplantation, there is currently no established serologic marker for noninvasive diagnosis of allograft rejection after human heart transplantation. Experimental noninvasive methods such as MRI (10), signal averaged ECG (11), specialized echocardiographic indices (12,13) or cytoimmunological monitoring (14) have been described; apart from being more technically demanding, less readily available and very costly, they currently do not offer sufficient diagnostic reliability, especially at later stages (>3 months) after heart transplantation.

Troponin T levels in graft rejection: cross-sectional analysis. The present study demonstrates that acute cellular graft rejections occurring more than 3 months postoperatively are

often associated with increased serum concentrations of the cardiospecific isoform of the myofibrillar protein troponin T. Troponin T serum levels and the proportion of samples above the diagnostic cutoff level rise in parallel with rejection severity (Fig. 1 and 2). Using the current detection limit of the assay (15 ng/liter) as the cutoff level, all cases of the most serious forms of allograft rejection (ISHLT grades 3B and 4) would have been detected at a presymptomatic stage. Based on the results of the cross-sectional analysis, the sensitivity and specificity of positive troponin T determinations for significant rejection (ISHLT grade 3/4) were 80.4% and 61.8%, respectively. More remarkable was, however, a negative predictive value of troponin T testing of 96.2%, suggesting that in the absence of detectable serum levels of troponin T significant allograft rejection is highly unlikely. An intraindividual longitudinal analysis of 15 patients during long-term follow-up confirmed these findings with further improved sensitivity and specificity (91.3% and 88.3%). In 7 additional cases elevated troponin T levels were found in severe immune vasculitis without myocardial involvement.

Pathomechanism. It appears most likely that this release of myocardial troponin T should originate from cardiac myocytes injured during the rejection process; a continuous release of troponin T over the first 2 to 3 months after heart transplantation has been described by our group (8). In experimental rat heart transplantation increased troponin T serum levels could be demonstrated in advanced graft rejection ([9] and our own unpublished observation), but the sensitivity of troponin T determination was limited by postsurgical troponin T release. Hossein et al. suggested the potential use of troponin T for noninvasive diagnosis of graft rejection (20). This study included only 6 patients, and the reported absence of elevated troponin T levels at the time of ongoing histological rejection is difficult to interpret. Wang et al. recently reported on the inability of myocyte death markers to predict cardiac allograft rejection (21); troponin T levels in rejectors were, however, indeed higher than in nonrejectors, though not statistically significantly. The present study thus appears to be the first demonstration of a consistent rise in the serum concentration of a cardiospecific marker during serious allograft rejection in a large series of patients after human heart transplantation.

In the cross-sectional and longitudinal analysis all cases of ISHLT grade 3B and 4 rejection would have been detected by troponin T testing before clinical signs of graft failure occurred. It is important to realize that according to a data analysis of the Cardiac Transplant Research Database only 3% of ISHLT grade 3B rejections and 8% of grade 4 rejections were associated initially with hemodynamic compromise or other clinical signs (22). Noninvasive monitoring by troponin T testing could be particularly valuable for the detection of allograft rejection at a stage beyond routine performance of endomyocardial biopsies.

Troponin T negative graft rejection. In a noninvasive screening test such as described here, false negative results are generally considered the most critical incidents, as true pathology is being missed. In the cross-sectional analysis of troponin

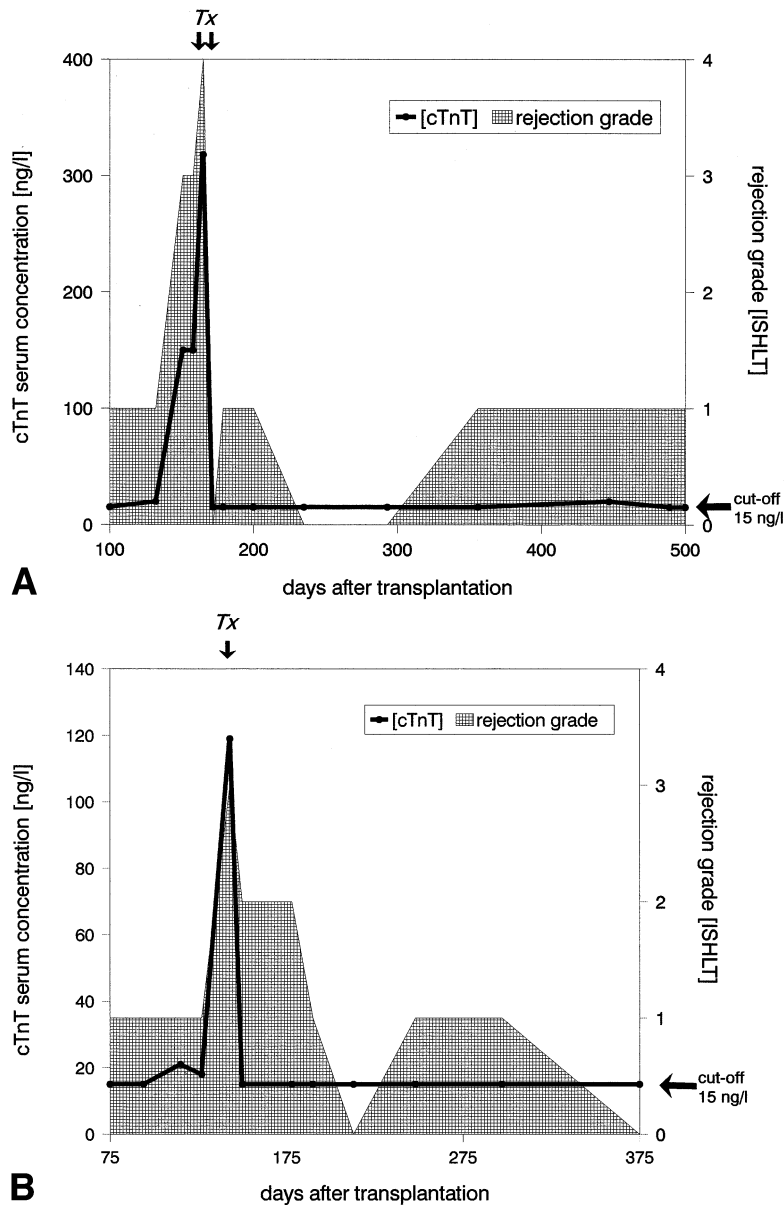


Figure 3. Longitudinal intraindividual analysis of troponin T concentrations (cTnT) and biopsy results. **A**, Patient EP, male, 48 years old. **B**, Patient WW, male, 42 years old. Tx, antirejection therapy.

T levels, 19.6% of more serious forms of allograft rejection (ISHLT grades 3 and 4) were not accompanied by a detectable increase in troponin T serum concentrations; all these incidents were limited to the lowest rejection grade within this subgroup, ISHLT 3A. In the intraindividual longitudinal analysis, this proportion was considerably improved, decreasing to only 2 of 23 cases (8.7%). As all these patients were clinically unremarkable at the time of rejection diagnosis, these incidents could represent early and still relatively mild rejection episodes, which might have become troponin T positive at a later stage. Troponin T negative forms of rejection might also be explained by the fact that myocardial cell damage during graft rejection occurs over a much more protracted period of time as compared, for example, to myocardial infarction or cardiac surgery. In contrast to animal experiments, the percentage of damaged myocardium in clinical graft rejection is

usually much smaller due to concurrent immunosuppression, limiting the release of troponin T. Thus, due to the short serum half-life of troponin T (10 min), significant serum concentrations may fail to develop, particularly in slowly progressing rejection episodes.

Elevated troponin T levels without rejection. The described troponin T test displays a comparatively high frequency of positive troponin T determinations, seemingly in the absence of underlying pathology (rejection, gross graft vascular disease, ischemic heart disease). This proportion of apparently false positive results reaches 25% already in the healthy control subjects and is not significantly increased in transplant patients showing no or only mild forms of rejection. Increasing the cutoff level did not significantly improve the specificity of the test, and it increases the risk of falsely negative results. False positive troponin T tests might be caused by undetected

ischemic events, for example, on the basis of transplant atherosclerosis, undetected ischemic heart disease (in the control subjects), intercurrent disease or other unknown factors. In fact, there was a trend for elevated troponin T levels without histological rejection to occur at later stages after transplantation in the longitudinal study, highlighting the potential influence of undetected graft vascular disease. The elevated troponin T concentrations in the cases of isolated stenosing immune vasculitis also underline the impact of microvascular lesions on troponin T release.

ISHLT grade 2 rejection. The data of this study may also contribute to the ongoing debate on the significance of ISHLT grade 2 rejection (23). Absolute troponin T levels are slightly, but not significantly, higher in grade 2 rejection than with no or grade 1 rejection. Thus, ISHLT grade 2 rejection might be considered a milder form of rejection similar to grades 0 and 1, not requiring therapy. The complex role of grade 2 rejection is, however, further illustrated by the 50% proportion of troponin T positive samples in cases of grade 2 rejection, which might warrant viewing grade 2 rejection as a separate intermediate form ($p < 0.02$ vs. ISHLT 1 and ISHLT 3A).

Longitudinal analysis. Sensitivity and specificity of troponin T testing were improved with intraindividual longitudinal analysis of samples. This may be accounted for by the selection of patients that had experienced several rejection episodes, therefore the biological severity (and the propensity for detectable troponin T release) may have been greater in these patients than in the cross-sectional analysis, despite the same histological diagnosis of ISHLT grade 3A rejection. Furthermore, continuous longitudinal monitoring allows the detection of a rejection episode at a somewhat later, but still clinically unremarkable, stage. Also, patients with constantly elevated troponin T levels, which can only be excluded in longitudinal monitoring, will increase the frequency of false positive results in cross-sectional studies.

Correlation with other clinical data. Apart from rejection severity, troponin T levels were not associated with any other clinical or laboratory parameters. Especially, no association with serum concentrations of the classic marker of myocyte damage, creatine kinase and its MB isoform, could be demonstrated, confirming previous findings (15–17). While no severe systemic infections were present among the study patients, minor infections and bacterial pneumonia in 3 cases did not affect troponin T concentrations in the absence of allograft rejection. The relatively large number of severe rejections at a later postoperative stage explains the lack of an inverse correlation of troponin T levels and time after transplantation. Troponin T release during some of these late rejections was similar to early postoperative rejections, suggesting that comparable myocardial injury can occur. Although an effect of impaired renal function on troponin T elimination has been demonstrated (5), renal function was not shown to influence troponin T serum concentrations in this study; however, patients with manifest renal failure (creatinine >4 mg/dl) had been excluded. Age, gender and preoperative diagnosis also had no effect upon troponin T levels.

Methodology/availability. In the present study an optimized troponin T ELISA was used with markedly improved sensitivity over currently available commercial assays (approximate factor 6–10). Current commercial assays use a diagnostic cutoff at 0.1 to 0.15 ng/ml (100–150 ng/liter). Only 13 of 185 positive samples (7%) in this study displayed concentrations above 100 ng/liter; a large proportion of positive samples would therefore have remained undetected by available commercial assays. In view of the proportion of positive samples among healthy controls (25.7%), a further increase in sensitivity appears unlikely to add to the diagnostic efficiency of troponin T testing.

The modified, noncommercial version of the troponin T used in this study is based on the Trop T-Enzymun test (Boehringer Mannheim, Mannheim, Germany). The necessary reagents can, however, be taken directly out of the commercial ELISA kit, and the necessary changes in the test protocol will easily be adopted by any clinical laboratory. The cost of a single troponin T determination currently comes to approximately \$10–20 per test.

Limitations. Limitations of the present study include the retrospective analysis and the overrepresentation of higher grade rejections by design, but the large number of samples and patients allow valid statistics. Use of the detection limit as cutoff level was taken into consideration by choosing a statistical approach underestimating differences between patient groups. Due to the retrospective approach and troponin T determinations only at the timepoint of endomyocardial biopsy, the course of troponin T concentrations in the period leading up to rejection cannot be evaluated. On theoretical grounds, however, the development of elevated troponin T levels during the induction phase of allograft rejection without myocardial cell damage appears less likely.

Future studies. To further assess the diagnostic efficacy of troponin T testing and confirm the present results more longitudinal data in individual patients will be required, ideally in a prospective and multi-institutional setting. Analysis of possible management decisions based on troponin T results will allow the quantitation of reductions in required biopsies and health care costs. The role of graft atherosclerosis on troponin T serum levels and potential diagnostic consequences will require further investigation. Troponin T levels in humoral rejection and the potential confounding influence of cytomegalovirus myocarditis cannot be addressed on the basis of the present data, since they were excluded from analysis. Also, the clinical significance of patients exhibiting continually elevated troponin T levels after transplantation needs to be elucidated further.

Conclusions and clinical implications. In summary, these data demonstrate that cardiac allograft rejection is often associated with increased serum concentrations of cardiac troponin T in a very sensitive assay, proving the feasibility of detecting cardiac graft rejection by an elevation of cardiospecific serological markers. All cases of the more severe forms of acute graft rejection would have been detected by troponin T determination before the occurrence of clinical symptoms.

Most remarkable, however, is the negative predictive value of troponin T testing of over 95%, supporting the assumption that in the absence of detectable serum troponin T levels the allograft is stable and significant rejection unlikely. Considering the confounding factors in the interpretation of troponin T test results, it becomes clear that this diagnostic method will not be applicable for every patient and that continuous longitudinal monitoring of troponin T levels will prove most reliable for diagnostic purposes. Even then, troponin T determination may not be suitable as a single noninvasive parameter for the diagnosis of graft rejection after heart transplantation. An important role of troponin T testing as an ancillary method for the exclusion of serious graft rejection during routine follow-up could, however, be envisaged, especially as potential alternative diagnostic modalities (MRI, specialized echocardiographic indexes, signal-averaged ECG, cytoimmunological monitoring) are considerably more technically demanding, costly and currently not widely available.

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